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Number and dimensions of rat glomerular capillaries in normal development and after nephrectomy

JENS RANDEL NYENGAARD

Stereological Research Laboratory, University Institute of Pathology and Second University Clinic of Internal Medicine, Institute of Experimental Clinical Research, University of Aarhus, Aarhus, Denmark

Number and dimensions of rat glomerular capillaries in normal development and after nephrectomy. Glomerular capillary growth was studied in kidneys in five- to 540-day-old perfusion-fixed normal or sham-operated rats (C) as well as in unilaterally nephrectomized three-day-old (NN) and 120-day-old (NA) rats. The number and volume of mature glomeruli were estimated using the fractionator. The glomerular number was unaffected by neonatal or adult nephrectomy, but the number of mature glomeruli in all rats aged five days ($19.1 \pm 2.0 \cdot 10^3$; ± SD) was significantly smaller than for all the older animals ($26.5 \pm 3.1 \cdot 10^3$). The mean glomerular volume increased 59% and 20% for the NN and NA rats, respectively, versus the C rats. A capillary unit has been defined according to the number of loops in the glomerular capillary network by the use of topology. Glomerular capillary number, estimated using a physical disector, increased 53% for NN rats and 26% for NA rats. The glomerular capillary length was estimated on isotropic, uniform random sections, and increased 47% for NN rats and 12% for NA rats. The glomerular capillary surface area increased 54% for NN rats and 14% for NA rats. The diameter of the glomerular capillaries increased 8% for the mature NN versus the C rats. The rather unexpected findings are discussed and related to interesting relationships, including the law of Poiseuille and LaPlace. In conclusion, the growth of glomerular capillaries after neonatal and adult nephrectomy is performed by branching that is making new glomerular capillaries, instead of simply lengthening the existing capillaries.

The extent of compensatory hypertrophy following unilateral nephrectomy is inversely dependent on the age of the rat at the time of nephrectomy [1]; younger rats have larger kidneys and greater glomerular filtration rate following nephrectomy [2, 3]. The total surface area and length of the glomerular capillary network are also increased in nephrectomized rats [4–6]. Whether the latter is caused by a lengthening of the existing capillaries or an increase in number of glomerular capillaries is, however, unknown.

To quantify total glomerular capillary number, a specific definition of a capillary unit is needed, which can be provided by topology that permits quantitation of changes of connections in complex three-dimensional networks. A capillary unit may then be given a topological definition, that is, Euler number or roughly speaking connectivity [7, 8], related to the development of a new capillary loop in the glomerular network: when the protrusion of existing endothelial cells confluences a close capillary segment [9], one new capillary loop has been generated. Similarly, the Euler number of the capillary network has changed by one.

The purpose of this study was therefore to establish if the glomerular capillary network adapts by dilating, lengthening or branching after neonatal and adult nephrectomy in rats by estimating the number, length, surface area and derived quantities of the hypertrophied glomerular capillary network using design-based stereological methods.

Methods

Animals

Wistar female rats from our own animal colony were allocated to neonatal unilateral nephrectomy, NN, or adult unilateral nephrectomy, NA, neonatal or adult sham-operation, or normal growth. Two neonatal and adult nephrectomized and two neonatal and adult sham-operated animals were chosen as well as one animal for normal growth for animals sacrificed at the same age. Operations on neonatal rats were performed three days after birth, whereas adult rats were four months on the day of the operation. The rats were weaned at day 30 and were kept in cages with a maximum of three animals per cage. The rats had free access to food (Altromin No 1324, Chr. Pedersen A/S, Ringsted, Denmark) and water.

Sham-operations and unilateral nephrectomies were performed under ether anesthesia. After a small flank incision was made, the right adrenal gland and renal capsule were separated from the right kidney. The right renal artery, vein and ureter were ligated at the renal pedicle, and the kidney was removed. Care was taken to avoid damaging the adrenal gland. The sham-operated animals were subjected to the same surgical procedure, except that the kidney and the renal pedicle were left undamaged.

Kidney fixation and preparation for light microscopy

The left kidneys in neonatal nephrectomized and sham-operated rats together with the controls were perfusion-fixed at days 5, 10, 30, 60, 135, 270, and 540 after birth. The adult nephrectomized and sham-operated animals had their kidneys perfusion-fixed at days 135, 270, and 540 after birth. The rats were anesthetized with pentobarbital (50 mg/kg) and were subjected to retrograde perfusion through aorta according to

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Maunsbach [10]. The infused solution contained 1% glutaraldehyde and 3% paraformaldehyde buffered in phosphate and was infused under constant pressure of 18.7 kPa for five minutes. Only the kidneys which blanched immediately were used. The kidneys were weighed after which they were coded in order to evaluate them blindly.

A tissue slicer [Fig. 24 in 11] was used for slicing the entire kidney. The thickness of the slices was constant from 0.5 mm to 1.5 mm according to the size of the kidney. Every third slice was sampled systematically random making three uniform sets of kidney slices. From the cortex of the first set of tissue slices, three blocks were sampled uniformly using a biopsy needle with a diameter of 1.5 mm and a plastic disc perforated with equidistantly spaced holes. The three tissue blocks were *en bloc* stained with uranyl acetate and embedded in Epon. Isotropic, uniform random sections were assured by the isector [12]. Three consecutive sections of thickness 1.5 μm were cut from each Epon block using a LKB Historange® microtome. The sections were stained with toluidine blue. The below-mentioned estimations on the Epon sections were evaluated as a ratio of weighted sums on blocks. The weights were the inverse sampling fraction of glomerular profiles in the middle section.

Another complete set of slices from each kidney was embedded in a single capsule using glycolmethacrylate (Historesin®). The entire plastic block was sectioned into 20-μm thick sections. In the larger animals, every sixth section (sampling section) together with the next section (look-up section) was sampled. In the smaller animals the sampling fraction was increased to one-third. One 2-μm thick section from the above-mentioned plastic block was needed for point counting. In order not to destroy the sampling scheme ten consecutive, 2-μm thick sections were cut instead of one 20-μm thick section, and one of the 2-μm thick sections was randomly sampled. All sections were stained with PAS.

Glomerular number and size

The total number of glomeruli in the kidney [N(glom)] was estimated with the fractionator [13] using two identical Olympus projection microscopes [Fig. 1 in 14] at a final magnification of 165×. A motor-driven microscope stage was mounted on one of the microscopes for sampling systematically random a known fraction of fields of vision in the series of 20-μm thick glycolmethacrylate sections. In a known fraction of the kidney, the glomeruli were only counted if they disappeared from the sampling section to the look-up section and vice versa (Q^-). The total number of glomeruli is then the number of counted glomeruli divided by the total sampling fraction.

$$N(glom) = \Sigma Q^- \cdot (\text{total sampling fraction})^{-1}$$

Mature or "filtering" glomeruli [15] belonging to the loop, the maturing, and the adult stage were counted [16, 17]. The below-mentioned 100× oil immersion objective was used to focus up and down in the 20 μm thick sections, when they contained glomerular profiles of an uncertain stage.

The mean glomerular size, $\bar{v}(glom)$, was estimated with a combination of fractionator sampling of glomeruli on the thick glycolmethacrylate sections, and point counting of glomeruli on the thin glycolmethacrylate sections. In detail, a point in the counting grid was used for sampling a fraction of cortex systematically random during glomerular counting (P_f). The counting grid represented a certain area of the section [frame area]. Section thickness (t) was 20 μm. P(glom) was the number of points hitting glomeruli whereas P(cor) was the number of points hitting cortex when moving the microscope stage with the thin glycolmethacrylate sections systematically random. The mean glomerular size:

$$\bar{v}(glom) = \frac{\frac{\Sigma P(glom)}{\Sigma P(cor)}}{\frac{\Sigma Q^-}{(\text{frame area}) \cdot t \cdot \Sigma P_f}}$$

Cortex was here defined as the volume of the kidney superficial to the arcuate arteries [18]. The size and number estimation of glomeruli is explained in detail in [19].

Estimation of number of glomerular capillaries

Using a low magnification of 165×, two glomerular profiles were sampled in the middle of the three consecutive Epon sections in animals older than ten days, whereas four glomerular profiles were sampled in the five and ten-day-old animals. To prevent sampling of incomplete and useless glomerular profiles at the edge, the glomerular profiles were located away from the edge at least one radius of the largest glomerular profile [20]. A 100× oil immersion objective was used to obtain a magnification of 1675×. Estimation of the area of the sampled glomerular profile [a(glo)], enclosed in a minimal string polygon [21] was done by point counting. The Euler number [χ(cap)] was estimated by comparing the sampled glomerular capillaries from a complete glomerular profile with the corresonding glomerular capillaries in the two neighbor sections (Fig. 1) using the two projection microscopes as a physical disector [22]. The middle section was used both as the sampled and the look-up section in relation to its two neighbor sections. The numerical capillary density in glomeruli [W_V(cap,glo)]:

$$W_V(cap/glo) = \frac{\Sigma\chi(cap)}{2 \cdot t \cdot \Sigma a(glo)}$$

t denotes thickness of the Epon sections. The calculation of absolute numbers of capillaries must take into account that the first capillary in a glomerulus connecting the afferent arteriole and efferent arteriole [9] is not counted as a loop. This vessel, of course, must be counted as one capillary. Moreover, one afferent and one efferent arteriole decrease the estimate by a number of one. The average total number of capillaries per glomerulus [$\bar{w}$(cap,glo)] is then:

$$\bar{w}(cap,glo) = [\bar{v}(glo) \cdot W_V(cap/glo)] + 2$$

The total number of glomerular capillaries per kidney, W(cap), is estimated by the multiplication of $\bar{w}$(cap,glo) with N(glo). The practical application and variability of the capillary number estimator has previously been described in detail [8].

Estimation of length and surface area density of capillaries

All measurements were performed on the above-mentioned sampled glomerular profiles in the isotropic, uniform random Epon sections at a magnification of 1675×. If Q_A denotes the

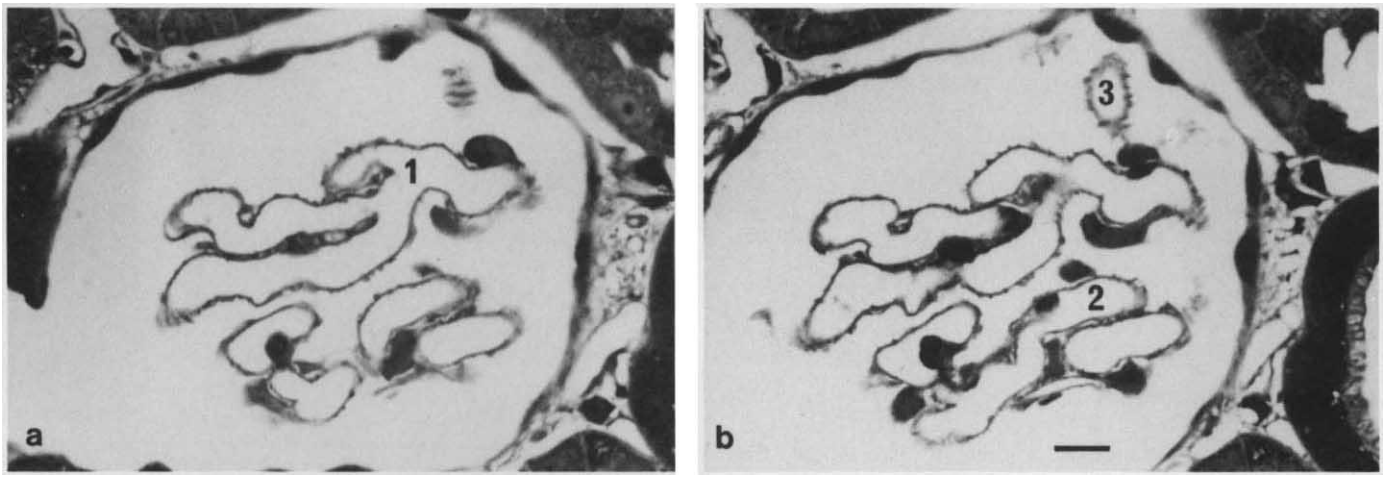

Fig. 1. *The contribution to the Euler number, χ, is shown using two adjacent Epon sections, stained with toluidine blue, from a rat glomerulus.* It should be emphasized that the observer looks for the topological events of the capillary *lumina*. In section a one capillary lumen (1) divides into three capillary lumina in section b giving rise to two "luminal connections". One capillary lumina (2) in section b divides into two capillary lumina in section a indicating one "luminal connection", and one capillary lumen (3) in section b is not seen in section a indicating one "luminal fragment". The rare "luminal lagoon", where an island of non-lumen appears inside the capillary lumen, is not seen here but has previously been shown schematically [Fig. 1 in 8]. All topological events of the capillaries were evaluated in the disector [22]. The contribution to the Euler number:

$$[\Sigma\chi(\text{cap}) = 1/2\cdot(\Sigma\#\text{luminal fragments} + \Sigma\#\text{luminal lagoons} - \Sigma\#\text{luminal connections})]$$

$\Sigma\chi$(cap) is used in the formula for estimating the numerical capillary density. The scale bar is 10μm.

number of capillary profiles divided by the glomerular profile area then the length density of capillaries in the glomeruli is:

$$L_V(\text{cap/glo}) = 2 \cdot Q_A$$

The surface area density of the capillaries in the glomeruli, S_V(cap/glo), was estimated as:

$$S_V(\text{cap/glo}) = 2 \cdot I_L$$

I_L denoting intersections between the test lines and the capillary surface area.

Number, length, and surface area of glomerular capillaries

To obtain the average total capillary length [$\bar{L}$(cap,glo)] and the average total capillary surface area per glomerulus [$\bar{S}$(cap, glo)] the length and surface area densities were multiplied with $\bar{v}$(glo). If $\bar{L}$(cap,glo) and $\bar{S}$(cap,glo) were multiplied with N(glo), the total length [L(cap)] and surface area [S(cap)] of glomerular capillaries per kidney were obtained, respectively.

Mean length, mean surface area, diameter, and cross sectional area of capillaries

These values were all calculated from the above-mentioned estimates. In detail, the mean length of capillaries [$\bar{l}$(cap)] was estimated as:

$$\bar{l}(\text{cap}) = \frac{L(\text{cap,glo})}{\bar{w}(\text{cap,glo})}$$

whereas the mean surface area of capillaries [$\bar{s}$(cap)] was obtained from

$$\bar{s}(\text{cap}) = \frac{S(\text{cap,glo})}{\bar{w}(\text{cap,glo})}$$

and with the assumption that capillaries are cylindrical tubes the mean diameter of capillaries, $\bar{d}$(cap), was derived from:

$$\bar{d}(\text{cap}) = \frac{\bar{s}(\text{cap})}{\pi \cdot \bar{l}(\text{cap})}$$

The mean cross sectional area of capillaries [$\bar{a}$(cap)] originated from the following equation:

$$\bar{a}(\text{cap}) = \frac{\pi \cdot \bar{d}(\text{cap})^2}{4}$$

Poiseuille's equation

The law of Poiseuille is strictly applicable only in tubes with fluids of constant viscosity and nonpulsatile, streamline flow. The flow of fluids (Q) through cylindrical tubes with a pressure drop (ΔP) due to resistance may then be described as [23]:

$$\frac{\Delta P}{Q} = 8 \cdot \pi \cdot \eta \cdot \frac{l(\text{cap})}{a(\text{cap})^2}$$

In this way a geometrical factor of the capillaries [l(cap)/a(cap)^2] comprising length [l(cap)], and cross sectional area [a(cap)] of a capillary tube is related to the function of the capillaries, flow and pressure drop via a constant. The constant is connected to the viscosity (η) of the fluid. The very complicated network of parallel and serial connected capillaries does not allow sampling of the correct average of l(cap)/a(cap)^2; therefore this report substituted the original geometrical factor with $\bar{l}$(cap)/$\bar{a}$(cap)^2. Although not all the above-mentioned assumptions are fulfilled with regards to the glomerular capillary flow, the law of Poiseuille gives the opportunity to relate the changes in geometry or "resistance" of glomerular capillaries to changes in the flow-pressure relationship in normal and nephrectomized rats.

Table 1. The coefficient of methodological error [CE_{ste} = SEM/$\bar{x}$] and the coefficient of observed interanimal variation [CV_{tot} = SD/$\bar{x}$] are shown for the stereological estimators of glomerular number [N(glo)] average total number of capillaries per glomerulus [$\bar{w}$(cap,glo)] average total length of capillaries per glomerulus [$\bar{L}$(cap,glo)] and average total surface area of capillaries per glomerulus [$\bar{S}$(cap,glo)]

	N(glo)	$\bar{w}$(cap,glo)	$\bar{L}$(cap,glo)	$\bar{S}$(cap,glo)
CE_{ste}	0.06	0.13	0.10	0.09
CV_{tot}	0.13	0.16	0.13	0.16

Statistics

This report estimates the variation of the stereological estimates at the level of blocks [24] as the coefficient of error (CE_{ste} = SEM/x). CE_{ste} of the number of glomeruli is estimated from the equations in Table 3 from West and Gundersen [25]. The capillary number, length, and surface area estimators are ratio estimators, therefore the formula given in Kroustrup and Gundersen [26] has been used for estimating their CE_{ste}. The total variation of the above-mentioned estimates (CV_{tot}) originates from the biological (CV_{bio}) and stereological variation: $CV^2_{tot} = CV^2_{bio} + CE^2_{ste}$. More details are given in [8].

Linear regression of the first kind has been employed to evaluate changes with age and the regression lines were compared according to Lentner [pp. 214-215 in 27]. An unpaired *t*-test was used to test differences between groups with respect to glomerular number and mean capillary length. The level of significance was 0.05.

Results

The sham-operated and the control rats are in the following called controls (C) because there was no difference between these two groups. Neither was there any difference between any groups of rats in body weight when sacrificed at the same age. The estimates of CE_{ste} and CV_{tot} for N(glo), $\bar{w}$(cap,glo), $\bar{L}$(cap,glo), and $\bar{S}$(cap,glo) are shown in Table 1.

There was no significant difference between the number of mature glomeruli (Fig. 2) in the NN group older than five days ($26.1 \pm 3.1 \cdot 10^3$; ± SD) and the controls ($26.7 \pm 3.4 \cdot 10^3$) older than five days ($2P = 0.62$). Neither was the number of mature glomeruli in the NA group ($26.4 \pm 2.3 \cdot 10^3$) different from the controls older than five days ($2P = 0.83$). The number of mature glomeruli was constant about $26.5 \pm 3.1 \cdot 10^3$ for all animals older than five days and therefore different from the number of mature glomeruli ($19.1 \pm 2.0 \cdot 10^3$) for the three C and two NN rats aged five days ($2P = 6.6 \cdot 10^{-6}$).

A significant regression of $\bar{v}$(glo) with age (Fig. 3, Table 2) was observed in the C and NN rats from 5 to 540 days ($2P = 2.2 \cdot 10^{-5}$ and $2P = 0.001$). The regression of the mean volume of glomeruli with age failed to be significant for the NA rats in the time interval from 135 to 540 days ($2P = 0.10$). The mean volume of glomeruli was 59% greater in the NN than in the C rats from 5 to 540 days ($2P = 2.5 \cdot 10^{-4}$). In the time interval from 135 to 540 days, $\bar{v}$(glo) was 37% greater in the NN rats than in the NA rats ($2P = 0.002$), and the NA rats had a 20% greater $\bar{v}$(glo) than the C rats ($2P = 0.02$).

The average total number of capillaries per glomerulus (Fig. 4; Table 2) regressed significantly with age in the time interval from 5 to 540 days in the C and NN rats ($2P = 0.006$ and $2P =$

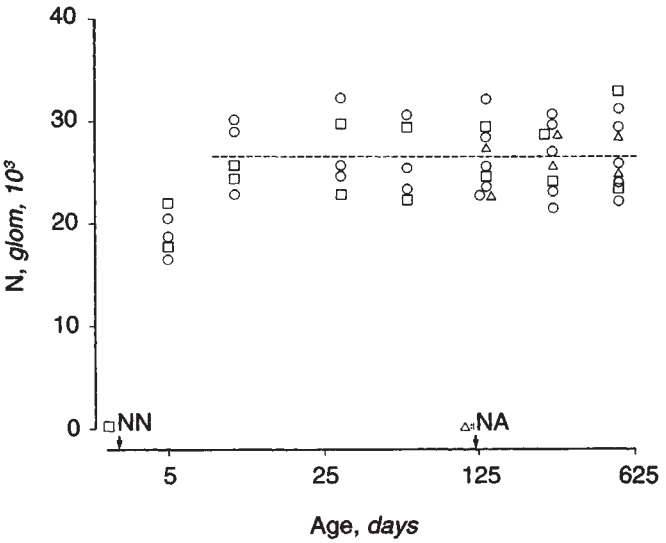

Fig. 2. *The number of mature glomeruli is shown for control (○), adult nephrectomized (△), and neonatal nephrectomized (□) rats.* The dotted line indicates the mean number of glomeruli for all animals older than five days (26500 ± 3100) which is significantly higher ($2P = 7 \cdot 10^{-6}$) than for the five days old animals (19100 ± 2000). NN indicates the date for neonatal nephrectomy and NA indicates the date for adult nephrectomy. The abscissa is logarithmic.

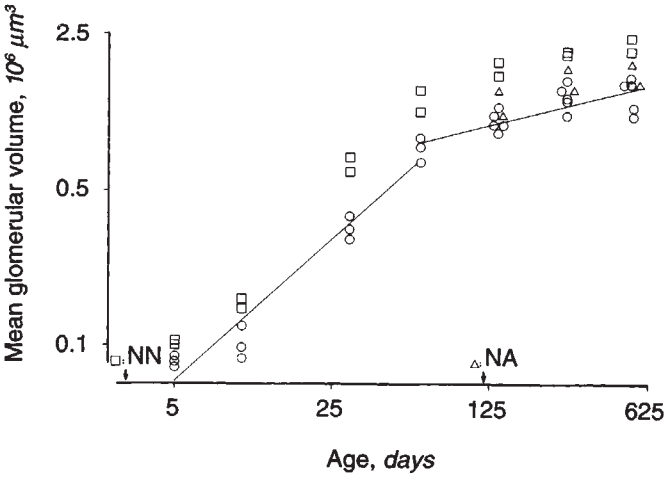

Fig. 3. *The mean volume of glomeruli is shown for control (○), adult nephrectomized (△), and neonatal nephrectomized (□) rats.* The two regression lines are for the control rats. NN indicates the date for neonatal nephrectomy and NA indicates the date for adult nephrectomy. The axes are logarithmic.

0.01). The regression of $\bar{w}$(cap,glo) from 135 to 540 days failed to be significant ($2P = 0.27$) in the NA rats. $\bar{w}$(cap,glo) was 53% greater in the NN than in the C rats from 5 to 540 days ($2P = 3.5 \cdot 10^{-4}$). The average total number of capillaries per glomerulus from 135 to 540 days was 23% greater in the NN rats compared with the NA rats ($2P = 0.02$), and in the same time interval the NA rats had a 26% greater $\bar{w}$(cap,glo) than the C rats ($2P = 0.01$).

The average total length of capillaries per glomerulus (Fig. 5, Table 2) in the C and NN rats regressed significantly with age from 5 to 540 days ($2P = 1.5 \cdot 10^{-4}$ and $2P = 0.004$). The

Table 2. The slopes and values of mean volume of glomeruli [$\bar{v}$(glo)], average total number of capillaries per glomerulus [$\bar{w}$(cap,glo)], average total length of capillaries per glomerulus [$\bar{L}$(cap,glo], mean cross sectional area of capillaries [$\bar{a}$(cap)], and average total surface area of capillaries per glomerulus [$\bar{S}$(cap,glo)], for control (C), neonatal nephrectomized (NN), and adult nephrectomized rats (NA)

			$\bar{v}$(glo)	$\bar{w}$(cap,glo)	$\bar{L}$(cap,glo)	$\bar{a}$(cap)	$\bar{S}$(cap,glo)
5–60 days	Log-log slope	C	0.92	0.94	0.96	0.35[a]	1.14
		NN	1.06	1.08	1.02	0.37[a]	1.20
10^{-9} μm/day				/day	mm/day	μm^2/day	10^{-3} mm^2/day
	Linear slope	C	12.6	2.47	0.13	—	2.30
		NN	19.3	3.72	0.19	—	3.42
	% Difference in position		C ⇔ NN 54%	50%	47%	1%[b]	51%
60–540 days	Log-log slope[c]	C	0.26	0.17	0.23	—	0.26
		NN	0.26	0.23	0.28	—	0.29
	% Difference in position		C ⇔ NN 64%	55%	46%	16%[d]	58%
Value at day 540					mm	μm^2	mm^2
$10^6 \mu m^3$		C	1.36	213	12.1	48.1	0.30
		NA	1.66	260	13.0	54.6	0.34
		NN	2.22	357	18.1	52.3	0.46

[a] Slope is different from 1.0 ($2P < 0.05$)
[b] Not statistical significant different from 0% ($2P > 0.05$)
[c] All slopes shown are different from 0 ($2P < 0.05$)
[d] Borderline statistical different from 0% ($2P = 0.06$)

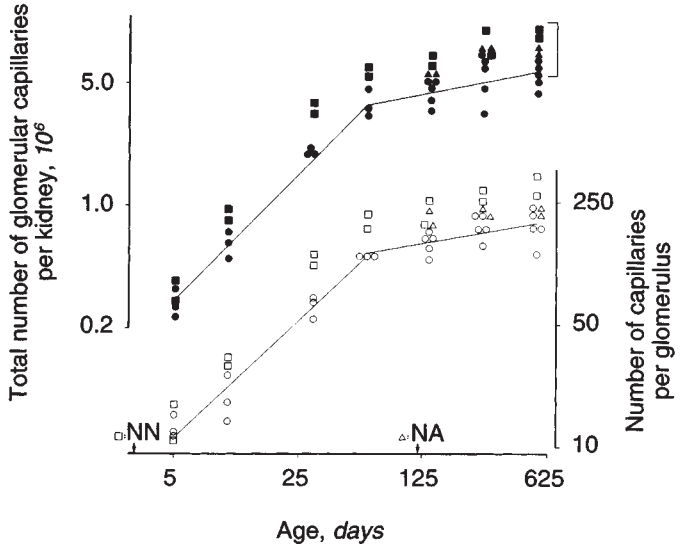

Fig. 4. *The average total number of capillaries per glomerulus and the total glomerular capillaries per kidney are shown for control (○ and ●), adult nephrectomized (△ and ▲), and neonatal nephrectomized (□ and ■) rats, respectively.* The four regression lines are for the control rats. The lower and upper horizontal lines of the bracket indicate the mean value of the total number of glomerular capillaries for one and two control kidneys aged 540 days, respectively. The total number of glomerular capillaries in two kidneys in a control rat exceeds the total number of glomerular capillaries in the remaining kidney in a neonatal unilaterally nephrectomized rat by 16%. NN indicates the date for neonatal nephrectomy and NA indicates the date for adult nephrectomy. The axes are logarithmic.

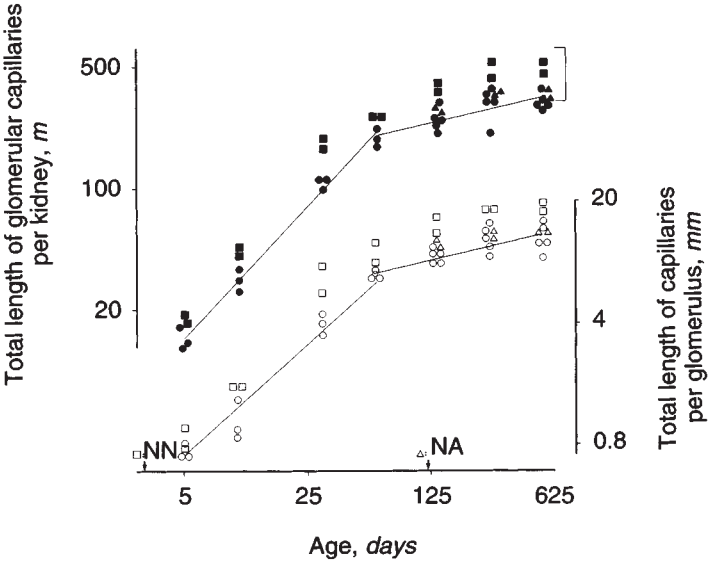

Fig. 5. *The average total length of capillaries per glomerulus and the total length of glomerular capillaries per kidney are shown for control (○ and ●), adult nephrectomized (△ and ▲), and neonatal nephrectomized (□ and ■) rats, respectively.* The four regression lines represent the control rats. The lower and upper horizontal lines of the bracket indicate the mean value of the total length of glomerular capillaries for one and two control kidneys aged 540 days, respectively. The total length of glomerular capillaries in two kidneys in a control rat exceeds the total length of glomerular capillaries in the remaining kidney in a neonatal unilaterally nephrectomized rat by 30%. NN indicates the date for neonatal nephrectomy and NA indicates the date for adult nephrectomy. The axes are logarithmic.

regression of $\bar{L}$(cap,glo) with age failed to reach the level of significance for the NA rats in the time interval from 135 to 540 days ($2P = 0.07$). $\bar{L}$(cap,glo) was 47% greater in the NN than in the C rats from 5 to 540 days ($2P = 5.7 \cdot 10^{-4}$). $\bar{L}$(cap,glo) from 135 to 540 days was 34% greater in the NN rats compared with the NA rats ($2P = 2.7 \cdot 10^{-4}$). In the same time interval, the difference in $\bar{L}$(cap,glo) between NA and C rats did not reach the level of significance ($2P = 0.13$).

A significant regression in the average total surface area of capillaries per glomerulus in the time interval from 5 to 540 days

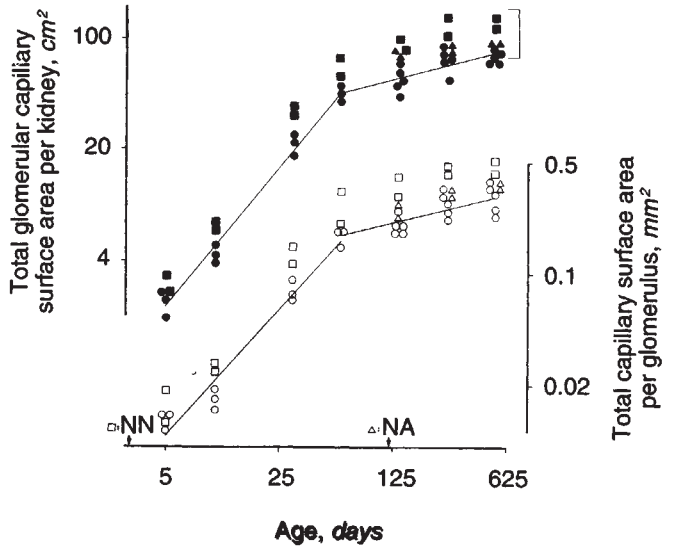

Fig. 6. *The average total surface of capillaries per glomerulus and the total surface of glomerular capillaries per kidney are shown for control (○ and ●), adult nephrectomized (△ and ▲), and neonatal nephrectomized (□ and ■) rats, respectively.* The four regression lines represent the control rats. The lower and upper horizontal lines of the bracket indicate the mean value of the total surface area of glomerular capillaries for one and two control kidneys aged 540 days, respectively. The total surface area of glomerular capillaries in two kidneys in a control rat exceeds the total surface area of glomerular capillaries in the remaining kidney in a neonatal unilaterally nephrectomized rat by 24%. NN indicates the date for neonatal nephrectomy and NA indicates the date for adult nephrectomy. The axes are logarithmic.

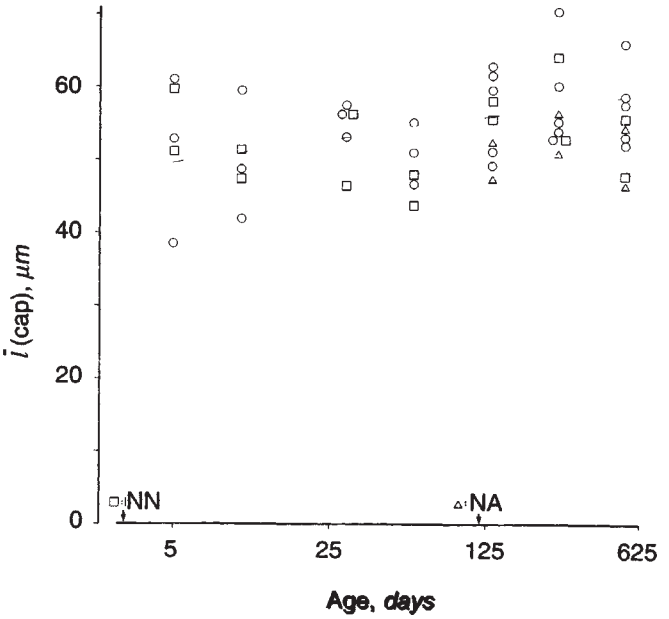

Fig. 7. *The mean length of glomerular capillaries is shown for control (○), adult nephrectomized (△), and neonatal nephrectomized (□) rats.* The dotted line represents the mean value for all rats (53.7 μm) which had a CV of 0.12. NN indicates the date for neonatal nephrectomy and NA indicates the date for adult nephrectomy. The abscissa is logarithmic.

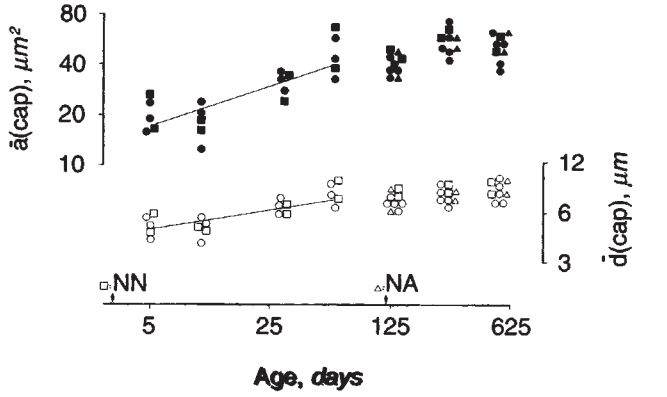

Fig. 8. *The mean diameter and the mean cross sectional area of glomerular capillaries are shown for control (○ and ●), adult nephrectomized (△ and ▲), and neonatal nephrectomized (□ and ⊡) rats, respectively.* The two regression lines represent the control rats. NN indicates the date for neonatal nephrectomy and NA indicates the date for adult nephrectomy. The axes are logarithmic.

(Fig. 6, Table 2) was observed in the C and NN rats ($2P = 3.9 \cdot 10^{-5}$ and $2P = 0.01$). The regression of $\bar{S}$(cap,glo) in the NA rats just failed short of statistical significance ($2P = 0.06$). $\bar{S}$(cap,glo) was 54% greater in the NN than in the C rats from 5 to 540 days ($2P = 1.7 \cdot 10^{-3}$). The average total surface area of capillaries per glomerulus from 135 to 540 days was 39% greater in the NN rats compared with the NA rats ($2P = 0.001$), and 14% greater in the NA rats compared with the C rats ($2P = 0.045$).

The mean length of capillaries in C rats (Fig. 7) regressed significantly with age ($r = 0.43$, $2P = 0.02$), however, the increase in $\bar{l}$(cap) from 5 to 540 days was only 18% (from 49.7 μm to 58.5 μm). The mean capillary length in the NN and NA rats did not regress significantly with age ($2P = 0.61$ and $2P = 0.81$, respectively). The mean length of capillaries in all the C rats (54.9 ± 6.9 μm) did not differ from $\bar{l}$(cap) in the NN rats (52.7 ± 6.2 μm) ($2P = 0.32$). In the time interval from 135 to 540 days, $\bar{l}$(cap) was significantly smaller in the NA rats (50.7 ± 3.4 μm) than in the C rats (57.6 ± 5.9 μm; $2P = 0.02$), whereas there was no significant difference between $\bar{l}$(cap) in the NN rats (55.9 ± 5.8 μm) and the NA rats ($2P = 0.09$).

The mean diameter and cross sectional area (Fig. 8, Table 2) regressed with age from 5 to 135 days with slopes of 0.18 and 0.35 in the C rats, and 0.19 and 0.37 in the NN rats ($2P = 0.001$ and $2P = 0.01$), whereas there was no significant regression from 135 to 540 days ($2P = 0.27$ and ($2P = 0.77$). There was no significant difference in $\bar{d}$(cap) and $\bar{a}$(cap) between C and NN rats from 5 to 135 days ($2P = 0.82$). The difference between C and NN rats from 135 to 540 days in $\bar{d}$(cap) and $\bar{a}$(cap) tended to be 8% and 16% ($2P = 0.06$). No significant difference was observed in the time interval from 130 to 540 days in $\bar{d}$(cap) and $\bar{a}$(cap) between the NA and C rats ($2P > 0.34$). The geometrical factor or "resistance" from the law of Poiseuille decreased with age in the NN, NA and C rats, but no difference between the three groups of rats can be observed (Fig. 9).

Discussion

This report has demonstrated an unexpected but significant increase in the number of topologically defined capillaries in neonatal and adult nephrectomized rats. Postoperative increase

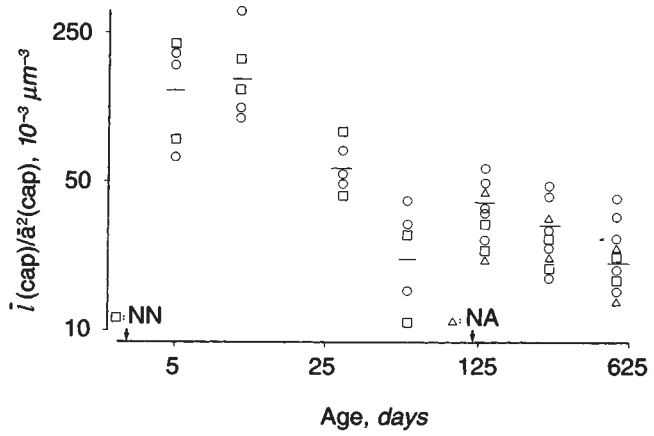

Fig. 9. *The "geometrical factor" or "resistance" from the law of Poiseuille is shown for control (○), adult nephrectomized (△), and neonatal nephrectomized (□) rats.* The mean value of resistance for the control rats is shown by the vertical lines. NN indicates the date for neonatal nephrectomy and NA indicates the date for adult nephrectomy. The axes are logarithmic.

in the number of glomerular capillaries was greater in NN than in NA rats as is usual for the age-effect on compensatory renal growth [1]. Formation of new capillaries in rat glomeruli has previously been found in normal growth [28]. Hypertrophied glomeruli in lithium-induced nephropathy [29] and in experimental diabetes [30] also generated new capillaries; on the other hand, small atubular glomeruli in lithium-induced nephropathy [29] had a smaller number of capillaries. Since the mean capillary length was reasonably similar for all rats, it is suggested that in general the variable in glomerular capillary growth is the number of capillaries. The complicated process of generating a new capillary loop instead of a simple lengthening of the existing glomerular capillary might serve a purpose: the formation of a complicated network of capillary loops instead of a bundle of parallel capillary loops has the advantage that the result of a blockade of a single segment will be reduced since the flow in the glomerular capillary network will be directed to other segments [31].

Hemodynamic effects of an increased number of glomerular capillaries after uninephrectomy cannot be assessed directly from this report. However, it is possible to indicate what might happen. The highly tentative and indirect calculation of the "resistance" of the glomerular capillary network suggests that uninephrectomy and the resulting increase in glomerular capillary number does not alter the resistance. The increase in glomerular capillary pressure gradient (ΔP) will be linearly related to the increase in the plasma flow (Q_A). This pressure gradient increase is, however, small compared to the pressure gradients across the afferent and efferent arterioles [32]. Since the cross sectional area of the glomerular capillary network is considerably greater than the cross sectional area at the afferent and efferent resistance sites, the glomerular capillary resistance contribution will be small and not have a significant impact on glomerular capillary pressure. The ultrafiltration coefficient (K_f), which is defined as the product of the hydraulic permeability of the capillary wall and total capillary surface area available for filtration per glomerulus, is presumably unaltered after uninephrectomy in mature rats [33], despite an increase in total glomerular capillary surface area. The considerable heterogeneity in capillary dimensions within a given rat glomerulus [34–36] is probably not decreased with the increased number of capillaries. Heterogeneity tends to reduce filtration efficiency of the glomerular capillary network [37, 38] because plasma is lead to short capillaries with low filtration fractions. Since the filtration fraction will decrease, the local rise in colloid osmotic pressure (π) will also decrease and cause the effective filtration pressure ($\Delta P - \pi$) to increase and moderate the decrease in filtration rate. This results in a Q_A related increase in single-nephron filtration rate [SNGFR = $Q_A \cdot (1 - C_A/C_E)$, where C_A and C_E are concentrations of protein in the afferent and efferent arteriole, respectively] without a significant change in either the ultrafiltration coefficient or glomerular capillary pressure gradient [SNGFR = $K_f \cdot (\Delta P - \pi)$] [33]. The influence of Q_A on SNGFR is based upon the rate of increase in π in the individual capillary segment. In multiple channel flow in capillary segments of unequal length, these equations have to be applied independently to each capillary segment. With many short capillary segments the filtration pressure equilibrium is less likely to be attained in the whole glomerulus, making it difficult for this model to visualize how SNGFR is increased without having hemodynamic and stereological data from each capillary segment.

The glomerular filtration rate in rats subjected to unilateral nephrectomy has been shown to increase about 40 to 60% above its preoperative value [33, 39], although the increase is greater in very immature animals [3]. The increase in mean or total glomerular volume has been found in some studies to be of the same magnitude as the increase in glomerular filtration rate [4, 5], although other reports have found a lesser increase [6, 40]. In this report, where estimation of mean glomerular volume was independent of the shape of glomeruli in contrast to the above-mentioned reports, a lesser increase has been found. Moreover, the postoperative increase in mean glomerular volume was greater in the NN than in the NA rats. The augmented glomerular sclerosis in aging rats following long-term uninephrectomy [41, 42], and especially following more extensive renal ablation [43–45], has been proposed to be the result of glomerular capillary hypertension. Recently, evidence has been put forward that glomerular hypertrophy was the important step preceding glomerulosclerosis [46–49]. Glomerular hypertrophy could in a synergistic way with glomerular capillary hypertension injure the glomerular capillaries by increasing the capillary wall tension as predicted by the law of LaPlace ($T = \Delta TP \cdot r$, T is wall tension, ΔTP is transmural pressure and r is capillary radius) [23]. One explanation could be that glomerular visceral epithelial cells were incapable of increasing their number and therefore were unable to regulate the permselective function of the hypertrophied glomeruli [50]. Bidani et al [45] and Daniels and Hostetter [51] found a 21 and 37% increase, respectively, in glomerular capillary radius in 5/6 nephrectomized rats. In contrast, neither Olivetti et al [4, 5] nor Shea, Raskova and Morrison [52] found an increase in capillary radius in uninephrectomized rats or in 5/6 nephrectomized rats, respectively. This report has only found an 8% borderline statistical significant increase in glomerular capillary diameter between mature NN and C rats, because capillary branching was the main response in compensatory glomerular hypertrophy. The law of

LaPlace may therefore only have limited importance in explaining why glomerular hypertrophy following uninephrectomy may accelerate the glomerulosclerosis seen in aging rats.

Autoradiographic studies have shown hyperplasia of glomerular cells in neonatal nephrectomy [53], whereas only hypertrophy of glomerular cells could be found in adult nephrectomy [53, 54]. Fries et al [50] found, however, evidence of hyperplasia of parietal epithelial cells, mesangial cells and endothelial cells, but not visceral epithelial cells in subtotal nephrectomy of mature rats. Apparently, mature rat glomerular endothelial cells can be induced to proliferate although their turnover rate is low.

In the 1970s there was a wide dispute about the influence of compensatory renal growth on glomerular number in rats. Four papers [55–58] found evidence of induction of new nephrons in young kidneys undergoing nephrectomy. It was peculiar that the first two cited papers found evidence of induction of new nephrons in hypertrophied kidneys even weeks after the end of nephrogenesis about seven to eight days after birth [59]. Two other reports from that period of time concluded that there was no increase in the number of glomeruli in immature kidneys undergoing nephrectomy [3, 60]. The latter two reports are in perfect agreement with the present report which gives the unbiased number of glomeruli [61].

In conclusion, this report has shown that rat glomerular capillaries grow by branching rather than lengthening or dilating in compensatory renal hypertrophy caused by neonatal as well as adult nephrectomy.

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Reprint requests to J.R. Nyengaard, Stereological Research Laboratory, Bartholin Building, Aarhus University, DK-8000 Århus C, Denmark.

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